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TREPONEMAL SURVIVAL IN HUMANS AFTER PENICILLIN THERAPY

A PRELIMINARY REPORT*

BY

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Collart, Borel, and Durel (1962a, b) reported research indicating the possible persistence of *Treponema pallida* in a group of humans and a group of rabbits after adequate treatment of late syphilis. They suggested that the presence of possibly attenuated organisms causes the persistence of immobilisins. Because of the important implications of this report and because a specific stain for *Treponema pallidum* in tissue had recently been developed in our laboratory (Yobs, Brown, and Hunter, 1964), further study of treated late syphilis in humans was undertaken.

Method

Patient Selection

102 prison inmate volunteers were screened on the basis of currently reactive treponemal immobilization (TPI) tests of their sera, completeness of history, and adequacy of treatment, as well as willingness to undergo lumbar puncture for cerebrospinal fluid examination and the surgical excision of an inguinal lymph node. Co-operation was required in compiling the most complete documentation of personal medical history possible, particularly as it pertained to the diagnosis and treatment of syphilis or other venereal diseases. By these criteria, 46 men were selected: 35 negroes, ten Caucasian men, and one Puerto Rican.

Although all their sera were reactive by qualitative TPI tests, the results of the VDRL slide and Kolmer Reiter protein tests varied widely, from non-reactive to strongly reactive (Table I). All the serological tests were performed by the Venereal Disease Research Laboratory of the United States Public Health Service, Chamblee, Georgia.

All but one man (Case 41) had had an adequate course of treatment, and most had been treated for syphilis repeatedly because of continued sero-positivity. From their histories it appeared that five (Cases 2, 15, 26, 28, 36) had first received adequate treatment for syphilis in the primary stage of the disease, six (Cases 17, 29, 38, 41, 43, 45) in the secondary stage, eleven in early latency, and nineteen in late latency. Three (Cases 7, 16, 30) had been treated for neurosyphilis, and two (Cases 22 and 23) for congenital disease. Eighteen had received only penicillin, and five (Cases 20, 32, 38, 43, 45) only arsenic and bismuth. Twenty had had courses of penicillin as well as courses of arsenic and bismuth. Arsenic, bismuth, and penicillin in combination comprised the initial treatment in two (Cases 30 and 34). Only one man, to be discussed later, had not been treated at all (Case 41).

Of the 45 men who had been treated, five (Cases 6, 19, 24, 25, 27) had received treatment in 1963 but not for the first time. None had had antibiotics less than one month before surgery. 28 had had at least one course of treatment between 1953 and 1963. Twelve had had no treatment for more than 10 years before this study.

Clinical Procedure

On the day of hospitalization, a complete physical examination was performed on each man, and the next morning a right inguinal node was removed under spinal anaesthesia. The excised nodes varied in size and consistency but were usually clinically palpable and measured up to $2 \times 0 \cdot 7$ cm. Some were tough and fibrous, others of normal consistency. Variations in size or consistency could not be correlated with history or later study findings. Cerebrospinal fluid obtained at the time of anaesthesia was tested for protein content, cell count, and VDRL slide test reactivity.

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Note: Trade names are for identification only and do not represent an endorsement by the US Public Health Service.

TABLE I

CLINICAL DATA FOR PATIENTS IN STUDY

Case No.	Age (yrs)	Race	First Diagnosis	First Adequate Treatment	Last Treatment for Syphilis	Antibiotics since Last Syphilis Treatment	Serum Tests			*Cerebrospinal Fluid		
							VDRL Slide		Kolmer Reiter Protein	VDRL Slide Test	Cell Count	Protein Content (mg. per cent.)
							Qualitative	Dil.				
1	43	Negro	Late latent	Pen.	1959 Pen.		WR	0	N	N	0	33
2	43	Negro	Primary	As, Bi	1961 Pen.		WR	0	R 4+	N	0	48
3	37	Negro	Late latent	As, Bi	1961 Pen.		WR	0	R 3+	N	0	40
4	38	Negro	Late latent	As, Bi	1961 Pen.		WR	0	R 4+	N	0	55
5	57	Negro	Late latent	Pen.	1961 Pen.		N	—	N	No tap		—
6	42	Negro	Late latent	Pen.	1963 Pen.		R	1	R 4+	N	0	30
7	57	White	Paresis	As, Bi	1961 Pen.		WR	0	R 4+	N	0	36
8	32	White	Late latent	Pen.	1955 Pen.	1961 Pen.	R	1	R 3+	N	Bloody tap	
9	40	Negro	Early latent	As, Bi	1961 Pen.		WR	0	N	N	Bloody tap	
10	**41**	**Negro**	**Early latent**	**1932 As, Bi**	**1960 Pen.**		**R**	**1**	**R 4+**	**N**	**0**	**40**
11	40	Negro	Late latent	As, Bi	1960 Pen.		N	—	N	N	0	29
12	**37**	**Negro**	**Early latent**	**1947 Pen.**	**1960 Pen.**		**WR**	**0**	**R 4+**	**N**	**0**	**51**
13	33	Negro	Late latent	As, Bi	1961 Pen.		WR	0	R 4+	N	0	43
14	32	Negro	Early latent	Pen.	1961 Pen.		R	4	R 4+	N	0	28
15	35	Negro	Primary	Pen.	1952 Pen.	1953 Pen.	WR	0	R 1+	N	0	38
16	51	White	Paresis	Malaria, As, Bi	1944 As (No Pen. Rx)		WR	0	N	N	0	32
17	33	Negro	? Secondary	Pen.	1962 Pen.		WR	0	N	N	Bloody tap	
18	48	Negro	Late latent	Pen.	1959 Pen.		WR	0	R 4+	N	1	48
19	30	Negro	Early latent	Pen.	1963 Pen.		WR	0	R 1+	N	0	36
20	**42**	**Negro**	**Late latent**	**As, Bi**	**1947 As, Bi**	**1960 Erythromycin 5 g. 1961 Pen.**	N	—	R 1+	QNS	0	27
21	53	Negro	Late latent	Pen.	1961 Pen.		WR	0	R 1+	QNS	0	50
22	**34**	**Negro**	**Congenital**	**As, Bi**	**1955 Pen.**		**R**	**4**	**R 3+**	**QNS**	**0**	**17**
23	29	White	Congenital	Pen.	1949 Pen.		WR	0	N	QNS	0	16
24	49	Negro	Late latent	As, Bi	1963 Pen.		R	8	R 3+	QNS	0	24
25	32	White	Early latent	Pen.	1963 Pen.		R	32	WR	N	0	32
26	34	Negro	Primary	As, Bi	1954 Pen.	1961 Pen. (adequate for syphilis)	WR	0	N	N	0	24
27	34	White	Late latent	Pen.	1963 Pen.		R	2	N	N	0	27
28	51	White	Primary	As, Bi	1962 Pen.		N	—	R 3+	N	0	52
29	**36**	**Negro**	**Secondary**	**1951 Pen.**	**1959 Pen.**	**1961 Pen.**	**WR**	**0**	**R 4+**	**N**	**0**	**41**
30	47	Negro	Asymptomatic CNS	As, Bi, Pen.	1957 Pen.		R	1	N	N	0	21
31	39	White	Early latent	As, Bi	1956 Pen.		WR	0	AC	N	0	43
32	46	Negro	Early latent	As, Bi	1940 As, Bi (No Pen. Rx)		N	—	R 1+	QNS	2	42
33	34	Negro	Early latent	As, Bi	1949 Pen.		N	—	WR ±	N	0	52
34	36	Negro	Late latent	As, Bi, Pen.	1948 Pen.	1962 Pen., Terramycin	N	—	R 4+	QNS	0	56
35	31	Negro	Late latent	Pen.	1956 Pen.	1958 Pen.	R	4	R 3+	N	0	33
36	45	Negro	Primary	As, Bi	1956 Pen.		WR	0	N	N	0	41
37	31	Negro	Late latent	Pen.	1948 Pen.		WR	0	R 1+	N	0	38
38	38	Negro	Secondary	As, Bi	No additional Rx		R	1	R 4+	N	0	35
39	46	Negro	Late latent	As, Bi	1947 Pen.		N	—	R 2+	N	4	43
40	43	Negro	Early latent	As, Bi	1939 Pen.		N	—	N	N	2	30
41	**22**	**White**	**Secondary**	**None**	**None**		**R**	**8**	**Not done**	**N**	**0**	**30**
42	57	Negro	Late latent	Pen.	1955 Pen.	1960 Pen. (1·8 m.u.)	WR	0	R 4+	N	1	52
43	43	Negro	Secondary	As, Bi	1939 As, Bi		N	—	N	N	1	25
44	43	Puerto Rican	Late latent	Pen.	1962 Pen.		N	—	R 1+	N	0	60
45	54	Negro	Secondary	As, Bi	1942 As, Bi		N	—	N	N	0	44
46	35	Negro	Early latent	As, Bi	1955 Pen.	1962 Terramycin 20 g.	R	1	R 2+	N	0	30

*Cerebrospinal fluid protein determinations performed using trichloracetic turbidimetric method given in USPHS "Serologic Tests for Syphilis", 1959 Manual. Normal range for this procedure is 15 to 55 mg. per cent.

AC = Anticomplementary WR = Weakly reactive R = Reactive N = Non-reactive QNS = Quantity not sufficient for testing

Virulence Studies

All extra-capsular tissue was removed from the freshly excised lymph node. One-third of the node was reserved for tissue studies; the remainder was immediately thoroughly macerated with a sterile mortar and pestle and the contents extracted in 1·5 ml. of a 1:1 mixture of sterile physiological saline with TPI-non-reactive rabbit serum. Equal amounts of approximately 0·4 to 0·5 ml. of the extract were injected into one testicle of each of two TPI-non-reactive rabbits. At the laboratory, all remaining fluid was used either for extensive dark-field examination or for smear preparation.

Each surviving recipient animal was followed at least 6 months. Testes were carefully palpated each week for 4 weeks. During the next 2 months, weekly testicular palpation was continued and inoculated testes were routinely aspirated bi-weekly for dark-field examination. In the interim and after 3 months, any suspicious lesion was also aspirated for dark-field examination. Serological testing was performed at these intervals: VDRL at 1½, 3,

and 6 months and TPI at 6 months. Whenever any inoculated animal became moribund, blood was drawn for VDRL and TPI testing; whenever possible, extracts of its popliteal nodes and, in some instances, testicular tissue were transferred to other TPI-non-reactive animals.

Microscopic Studies

Smears of inoculating suspension, as well as sections and impression smears of the intact fragments of nodes, were stained by the silver impregnation method of either Dieterle (1927) or Krajian (1939), and by the direct fluorescent antibody (FA) technique (Yobs and others, 1964). Each node was studied as follows:

For 2½ hours by dark-field examination of inoculating suspension;

For 2½ to 3 hours by examination of fluorescent antibody sections and smears;

For 4 hours by examination of silver-stained sections and smears.

This involved a minimum of 9 hours' meticulous microscopic study.

Results

Rabbits receiving tissue from two patients (Cases 10 and 12) developed dark-field positive testicular lesions. *T. pallidum* was demonstrated only in the node of Case 12 by fluorescent antibody staining (Table II).

TABLE II

LABORATORY FINDINGS IN EXCISED INGUINAL LYMPH NODES BEFORE TREATMENT

Case No.	Inoculation Suspension		Stained Sections		Dark-field Positive Testicular Lesion in Rabbit Recipient
	Dark-field	Fluorescent Antibody	Fluorescent Antibody	Silver	
10	—	—	—	—	+ Day 29 + Day 35
12	—	—	+	—	+ Day 36 + Day 36
20	—	+	—	—	—
22	—	+	—	—	—
29	—	—	—	+	—
41	—	—	—	—	—

However, in nodes from two other patients (Cases 20 and 22), *T. pallidum* was demonstrated by FA without the development of disease in parallel animals. In one of these, Case 22, prolonged dark-field examination of inoculating suspension repeated 8 hours after the excision of the node revealed two non-motile spiral organisms morphologically consistent with *T. pallidum*. In a fifth donor's node (Case 29) only one silver-stained section showed spiral organisms morphologically resembling treponemes; again, the rabbit recipients were negative (see Figs 1 to 4).

At the present time, 6 months' observation has been completed on the survivors of the 92 rabbit recipients. Data for the rabbit recipients of nodes found positive are shown in Table III.

TABLE III

RABBIT RECIPIENT DATA

Case No.	Rabbit No.	Rabbit Testis Dark-field	Serum Tests (mths)			
			VDRL			TPI
			1·5	3	6	6
10	909	+ Day 35	R 2·5 dil.	R 1 dil.	R 2·5 dil. R 1 dil.	N
	914	+ Day 29	N	R 25 dil.	Died	—
12	911	+ Day 36	N	N	N	N
	912	+ Day 36	Died at 5 wks			
20	949	—	N	N	N	N
	953	—	N	N	N	N
22	956	—	N	N	N	N
	959	—	N	N	N	N
29	969	—	N	N	N	N
	970	—	N	N	N	N

VDRL = Venereal Disease Research Laboratory
R = Reactive
N = Negative
TPI = Treponema pallidum immobilization

CASE HISTORIES

The case histories of the five men found positive by at least one study method are presented in greater detail in Table IV.

Case 10, a 41-year-old Negro, was first treated in 1932 with arsenic and bismuth. He was treated frequently thereafter and had received a total of 19·8 million units penicillin, the last recorded in 1960.

Case 12, a 37-year-old Negro, received penicillin in 1947 because of reactive serology; his serology in 1944 had been negative. In 1948, he developed tachycardia with dyspnoea; he was told this was caused by his thyroid gland and therefore underwent thyroidectomy. In 1954, in view of positive serological tests, he was given 14 g. each of Terramycin and Aureomycin. A diagnosis of syphilitic heart disease was made in 1960, when he took only seven of ten scheduled daily injections, 600,000 units aqueous procaine penicillin G. Examination on hospitalization for this study showed cardiomegaly, a pulse pressure of 75 with diastolic pressure of 65, and a loud diastolic murmur of aortic insufficiency.

Case 20, a 42-year-old Negro, was treated 2 years after the appearance of the primary lesion. Although he received antibiotics for other reasons, he was never treated for syphilis with penicillin.

Case 22, a 34-year-old Negro, had been treated with arsenic and bismuth for congenital syphilis until he was 13 years old, when he first received penicillin. From 1951 to 1961, he was given a minimum of 36 million units penicillin.

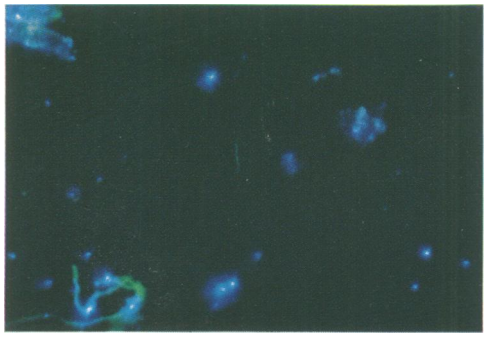

FIG. 1.—Smear of extract of lymph node from Case 12 stained by direct fluorescent antibody technique, showing one *T. pallidum* in centre. × 1080.

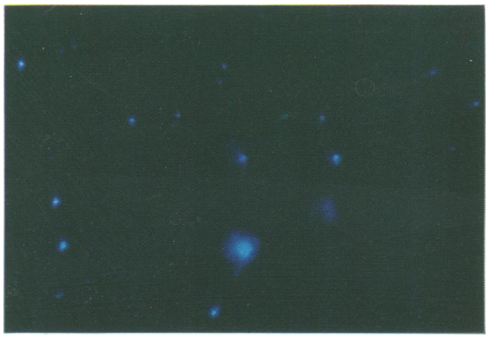

FIG. 2.—Smear of extract of lymph node from Case 12 stained by direct fluorescent antibody technique, showing three *T. pallida* in centre area. Two are entwined, while the third is apart. × 900.

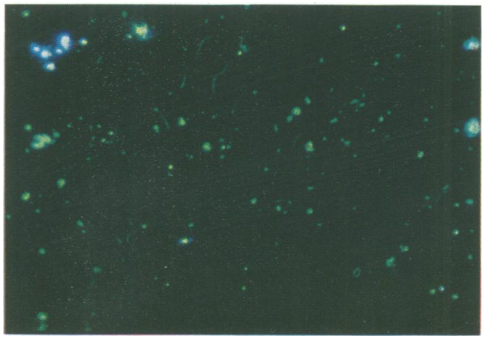

FIG. 3.—Fixed smear of extract of lymph node from Case 20, showing *T. paliidum*. Slide originally FA-stained, but fluorescence had been "burned out" before photograph could be made. × 1080.

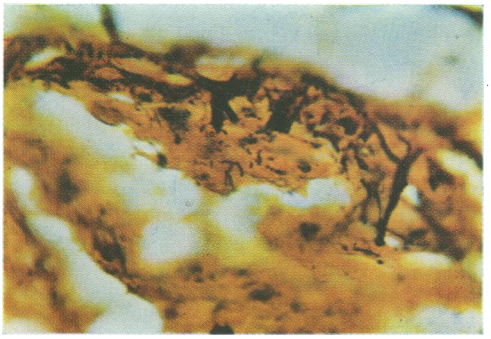

FIG. 4.—Section of lymph node from Case 29 stained by Dieterle's silver impregnation technique, showing spiral forms morphologically resembling *T. pallidum*. × 1900.

TABLE IV

CLINICAL DATA ON TREATED PATIENTS FOUND POSITIVE IN LABORATORY STUDY OF NODES

Case 10. 41-yr-old Negro	Case 12. 37-yr-old Negro	Case 20. 42-yr-old Negro	Case 22. 34-yr-old Negro	Case 29. 36-yr-old Negro
1932—Sero-reactive. Rx "hip and vein" shots 1945—Sero-reactive. Rx not identified 1949—Sero-reactive. Rx penicillin; dose unknown 1950—Rx PAM 3M units 1951—Rx PAM 3M units 1952—4+ 4 dil.; 4+ 16 dil. Rx PAM 3·6M units 1954—4+ 4 dil. 1956—4+ 8 dil. Rx PAM 3M units 1957—Rx PAM 1·2M units 1958—"Negative" 1960—VDRL 1:4. Rx APPG 6M units 1963—TPI R; VDRL R-1; KRP 4+ CSF protein 40 mg. per cent.; cells 0; VDRL negative Rabbit recipients +	1944—Kahn and Kolmer negative 1947—Sero-reactive. Rx APPG 6M units 1948—"Heart trouble" with shortness of breath No history rheumatic fever. Thyroidectomy 1954—Flocculation test R 1:2 Complement-fixation test positive Terramycin 250 mg. and Aureomycin 250 mg. four times daily for 2 weeks 1955—Flocculation test negative Complement-fixation test doubtful 1960—VDRL R 1:2 Diagnosis luetic heart disease Rx APPG 6M units ordered, but patient took only 4·2M 1963—TPI R; VDRL WR-0; KRP 4+ CSF protein 51 mg. per cent.; cells 0; VDRL 0 Rabbit recipients and fluorescent antibody sections +	1945—Penile chancre Healed spontaneously without treatment 1947—Sero-positive As, Bi twice weekly for 19 wks Kolmer doubtful; Kahn positive 1948—Kahn negative 1960—Erythromycin 250 mg. four times daily for 5 days for non-syphilitic infection 1961—Penicillin for cold, 1 shot 1963—TPI R; VDRL negative; KRP 1+ CSF protein 27 mg. per cent.; cells 0; VDRL QNS Fluorescent antibody node suspension positive	Congenital syphilis treated with bismuth and arsenic until 13 years old, when penicillin was given 1951—4+ 1:2 Rx APPG 6M units three times 1952—Rx APPG 6M units 1953—Rx APPG 6M units 1954—4+ WP undil. 1955—4+ P undil. Rx APPG 6M units 1961—VDRL R 1:4 1 "big" shot procaine penicillin G. Kolmer R; VDRL 1:1 1963—TPI R; VDRL R 4 dil.; KRP 3+ CSF protein 17 mg. per cent.; cells 0; VDRL QNS Fluorescent antibody node suspension positive	1945 and 1949—sero-negative 1950—Diagnosis: Primary syphilis Rx PAM, 2 shots 1951—Genital lesion and skin rash; multiple moist rosette lesions of glans and prepuce; mucous patch of soft palate dark-field positive VDRL 1:128 CSF protein 20 mg. per cent. Rx 4·8M units penicillin in one dose 1952—VDRL 1:64. Rx PAM 3M units 1953—4 dil. Rx PAM 3M units 8 dil. Rx PAM 3·6M units 1954, 1955—2 dil. 1956—VDRL WR; Kolmer 4+ 1957—Rx PAM 3M units, one dose 1959—Rx APPG for 6 days 1961—Rx APPG 600,000 units and Bicillin 600,000 units for pharyngitis 1963—TPI R; VDRL WR 0 dil.; KRP 4+ CSF protein 41 mg. per cent.; cells 0; VDRL negative Silver-stained section positive

QNS = Quantity not sufficient for testing
APPG = Aqueous procaine penicillin G
PAM = Penicillin aluminium monostearate
TPI = Treponemal immobilization test
VDRL = Venereal Diseases Research Laboratory
KRP = Kolmer Reiter protein test
R = Reactive
WR = Weakly reactive
WP = Weakly positive
P = Positive

Case 29, a 36-year-old Negro, had a primary infection in 1950, and secondary manifestations appeared in 1951; he had had 23·4 million units penicillin, the last in 1959.

One other patient should be mentioned.

Case 41, a 22-year-old white male, supposedly developed a genital lesion suggestive of a chancre one week after possible exposure; this healed without treatment. When a medical history was taken on imprisonment 2 to 3 months later, he complained of "haemorrhoids", but rectal examination was not recorded. One month after imprisonment he volunteered for this study, denying any history of chancre or previous venereal infection. Upon hospitalization for this study, three perianal condylomata lata were found; these were teeming with highly motile *T. pallida*. Some 18 hours later, dark-field examination of the lesions showed numerous non-motile spiral forms consistent with *T. pallidum*. The patient admitted only having washed with germicidal soap 2 hours earlier. Serum testing for penicillin was negative, but it is considered possible that some other antibiotic may have been obtained through illicit channels for self-administration. It was decided that, although this patient had untreated secondary syphilis, he would continue in the project, hopefully as a positive control, and a node was removed as planned. Examination of the node and inoculating suspension gave negative results. No dark-field positive lesions were noted in the recipients, but one of the two recipients was VDRL and TPI-reactive at 3 and 6 months. Lesion exudate obtained on the day of surgery infected the only animal inoculated with it (Table V).

Because of frequent inconsistencies in historical data and because we knew that this population, although "closed", still offered an opportunity for venereal infection, we requested expert assistance in

TABLE V

DATA ON ANIMAL RECIPIENTS OF MATERIAL FROM CASE 41 (UNTREATED SECONDARY SYPHILIS)

Patient Material	Rabbit No.	Dark-field Lesion in Testis	Serum Tests (mths)				
			VDRL			TPI	
			1·5	3	6	3	6
Node	993*	—	Not done	R 10 dil.	R 5 dil.	R	R
Node	997	—	Not done	N	N	N	N
Lesion Exudate	996	+ 31 Days	No serological tests performed				

* Subtransfer of popliteal nodes at 6 mths produced dark-field positive syphilis in one recipient after 45 days.

obtaining epidemiological information. Only one man, Case 20, admitted homosexual activity while in prison, but the information available did not permit the positive identification of contacts.

These six men (Cases 10, 12, 20, 22, 29, and 41) have now been treated under supervision (Table VI); 3 months after completion of treatment, a left inguinal node was removed under local anaesthesia from Cases 10, 12, 20, and 22, and these were studied in the laboratory as before, all results being negative.

A second node was not removed from Case 41 because of the stage of the disease at treatment.

TABLE VI
TREATMENT ADMINISTERED UNDER SUPERVISION

Case No.	Dosage
10, 22, 29	2·4M units Bicillin intramuscularly wkly for 2 wks (1·2M units into each gluteal muscle) Total 4·8M units
12	2·4M units Bicillin intramuscularly wkly for 3 wks (1·2M units into each gluteal muscle) Total 7·2M units
20	Erythromycin 500 mg four times daily for 15 days Total 30 g.
41	2·4M units Bicillin intramuscularly (1·2M units into each gluteal muscle) Total 2·4M units

M = Million

Case 29 is still in the post-treatment follow-up period. Rabbit recipients have been negative during 4 months' observation and serological testing, and the 6 months' observation has not been completed.

Comparison with Collart's Findings

To summarize, virulent *T. pallidum* was demonstrated in only two (Cases 10 and 12) of our 45 patients who had been treated before this study, and the adequacy of treatment of Case 12 may be questioned. In two others (Cases 20 and 22), *T. pallidum* was demonstrated by specific histochemical (FA) staining; Case 20 admitted homosexual activity in prison. A fifth man (Case 29) was found positive for treponeme-like organisms by a non-specific (silver) stain.

Nodal tissue from Case 41, with untreated secondary syphilis, produced both VDRL and TPI reactivity in one rabbit recipient at 3 months; subtransfer from this animal produced dark-field positive lesions.

In comparison, Collart and others (1962) reported studies of inguinal nodes from nine patients who had been treated for late latent or neurosyphilis (Table VII); they found organisms which they considered to be *T. pallidum* in silver-stained smears from all nine nodes. Animal inoculation was attempted in six: one was negative at 3 months, two were still under observation at the time of the report, and in three, lesions developed in which they observed spiral organisms by silver-stained smear at 42, 54, and 113 days—these they considered to be *Treponema pallidum*. No follow-up serological data on these animals were reported. On this evidence they considered three of their patients to harbour organisms capable of producing the disease.

Discussion of Laboratory Techniques

Each procedure in this study had its limitations. Some of the problems encountered had not been anticipated. Accurate documentation of diagnosis and treatment history was not possible in some instances because older records had not been preserved, pertinent events having occurred 10, 20, 30, or more years ago, and because identification of specific patients was impossible with incomplete vital statistics.

Three laboratory methods were used to demonstrate micro-organisms, especially *T. pallidum*, in nodes:

Dark-field microscopy is the oldest and most widely accepted. Absolute identification depends on the demonstration of morphology and motility typical of the organism and on the expertness of the microscopist. Results with this procedure were consistently negative. In one case, after *T. pallidum* had been found on FA stained suspension smears, re-examination of similar material from the same patient some 6 to 8 hours after surgery showed two non-motile spiral forms morphologically resembling *T. pallidum*.

TABLE VII
SUMMARY OF RESULTS (COLLART AND OTHERS)

Case No.	Age (yrs)	Stage of Syphilis	Treatment	Smear Reports	Animal Inoculum
1	66	Late latent	As, Bi	Typical *T. pallidum*	Not done
2	65	Late latent	As, Bi, Pen.	Atypical *T. pallidum* (doubtful)	3 mths negative
3	75	Tabes	Few injections (? type)	Typical *T. pallidum*	Not done
4	38	Rx'd Tabes	Bi, Pen.	Typical *T. pallidum*	Under observation
5	59	Rx'd Tabes	As, Hg, Pen.	Atypical *T. pallidum*	113 days + —atypical
6	56	Rx'd Tabes	Bi, Pen.	Typical *T. pallidum* (19 day) (Post-Pen.)	54 days + —atypical
7	64	Rx'd Tabes	As, Bi, Hg, Pen.	Atypical *T. pallidum*	42 days + —typical
8	49	Rx'd Tabes	Bi, Hg, Pen.	Typical *T. pallidum*	Under observation
9	46	Rx'd G.P.I. and Tabes	As, Bi, Pen., malaria, cortisone	Typical *T. pallidum*	Not done

Rx'd = Treated

Fluorescent antibody staining is the newest and most specific staining method available to-day, depending on an antigen-antibody reaction for specificity. Edwards (1962) reported using indirect fluorescent antibody staining of lesion exudate to diagnose syphilis. Yobs and others (1964) reported the application of indirect and direct staining to tissues; this procedure is extremely sensitive, requiring only a small amount of labelled antibody for staining. *T. pallidum* was shown by this technique in nodes from three men, and one of these nodes also infected animals.

Silver stains are non-specific; examining as many as twelve to fifteen preparations per node, spirochaetal forms were found in only one by this method.

Animal inoculation is considered by many to give uncontestable results. However, human isolates are thought to require an acclimatization period upon initial transfer to rabbits. The inoculation of tens of thousands of *Treponema pallida* may not infect every subject. Four of 92 rabbits developed dark-field positive lesions 29 to 36 days after inoculation. Serological data on these animals are not consistent. Two became VDRL-reactive but remained TPI-non-reactive; the other survivor of the four was TPI-non-reactive at 6 months. Nodal tissue from one of these VDRL-reactive, TPI-non-reactive recipients produced dark-field positive lesions at 17 days when subtransferred 3 months after the original inoculation. All but one of the other surviving animal recipients were TPI-non-reactive at 6 months; this one was the recipient of nodal tissue from Case 41, which has been discussed.

Use of the infectiousness of nodal tissue from one body area as a determinant of the presence of a few *Treponema pallida* anywhere in the body is not 100 per cent. sensitive. Failure to demonstrate a spirochaete by known techniques in any appreciable volume of specimen, even after hours of meticulous searching, does not rule out their presence. Each lymphatic drainage area includes a large number of lymph nodes. We attempted to examine only one. It is conceivable that while the nodes studied were negative, others may have been positive. It is also possible that treponemes were present but not seen.

Effectiveness of Penicillin in Antisyphilitic Therapy

It is essential that any possibility of re-infection be ruled out before these data can be interpreted as signifying the survival of virulent *T. pallida* in humans following adequate treatment. The population from which volunteers were obtained for this study, although closed, was not excluded from possible re-infection. Also, many of the patients had been at liberty for various intervals since their last course of treatment. 3 months after supervised re-treatment, an additional node removed from each of four men initially positive was non-infectious and negative by all procedures.

Our findings to date do not minimize the ability of penicillin to eradicate organisms capable of producing syphilis when it is administered according to recommended treatment schedules. This study has not explained the presence of spiral organisms in several of the individuals discussed who had allegedly received much penicillin. These spiral organisms morphologically resemble *Treponema pallida*, and some were pathogenic for rabbits. Re-infection must be considered as a possibility in the light of the negative results in nodes removed after the supervised administration to these patients of currently accepted adequate treatment. Furthermore, in 20 years of using penicillin in treatment of syphilis, no information has been obtained which suggests that the disease cannot be cured or arrested, regardless of stage or duration of infection at the time of adequate therapy.

Summary

Inguinal nodes of 45 men who had been treated for syphilis at various stages of the disease were studied. Five nodes were shown to contain treponemal forms, and in two cases these were shown to be virulent. Findings were completely negative in an additional node removed from each of four men after supervised treatment. The possibility of re-infection must be absolutely ruled out before these findings can be interpreted as demonstrating treponemal survival in humans after adequate penicillin treatment.

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Survie du tréponème chez l'homme après traitement par la pénicilline

RÉSUMÉ

On étudia les adénopathies inguinales de 45 hommes traités pour syphilis à différents stades de la maladie. On prouva que 5 ganglions contenaient des tréponèmes et que dans deux cas ils etaient virulents. Les résultats furent complètement négatifs lorsqu'on enleva un autre ganglion, chez 4 hommes après un traitement contrôlé. On doit éliminer complètement la possibilité de réinfection avant d'interpréter ces résultats comme démontrant l'existence de tréponème chez l'homme après un traitement correct par la pénicilline.